Analysis of persistence and effectiveness of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* IR27 inoculated on jujube trees (*Ziziphus mauritiana* Lam.) in the field, using the *RPB1* gene marker and measurement of fruit yield

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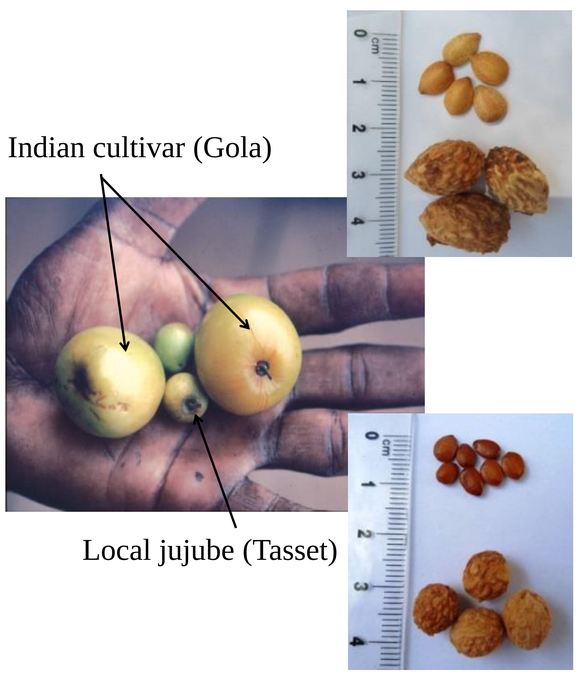
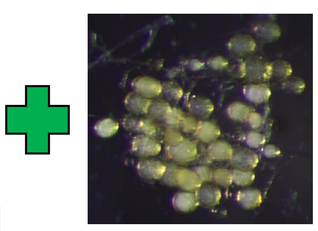
Ziziphus mauritiana Lam.

Ziziphus mauritiana Lam., commonly named jujube, is a multipurpose fruit tree well adapted and commonly used (eg. fruits, fodder) by local inhabitants in Sahelian and Sudanian areas in West Africa [1]. West African farmers are interested by Indian cultivars because of their precocity in fruiting, the larger size of their fruits and their taste [2, 3]. Furthermore, the orchards in its zone were found to be deficient in P, one of the most important soil factors determining productivity and quality of jujube fruits. Until now, the success of mycorrhizal plants has been based on plants inoculated with the *Rhizophagus irregularis* isolate IR27 under nursery conditions, while AMF inoculation has received little attention in the field [4]. The aim of our study was to evaluate the persistence and effectiveness of the *R. irregularis* isolate IR27 on growth and fruit yield of two provenances of jujube (Tasset from Senegal and Gola from India) in nursery and field conditions.

Jujubes

Experiment in greenhouse conditions





Rhizophagus irregularis IR27

Z. mauritiana (Gola) at 4 months after sowing

Effect of inoculation with *R. irregularis* IR27 on growth and mycorrhizal colonization of *Z. mauritiana* provenances at 4 months after sowing

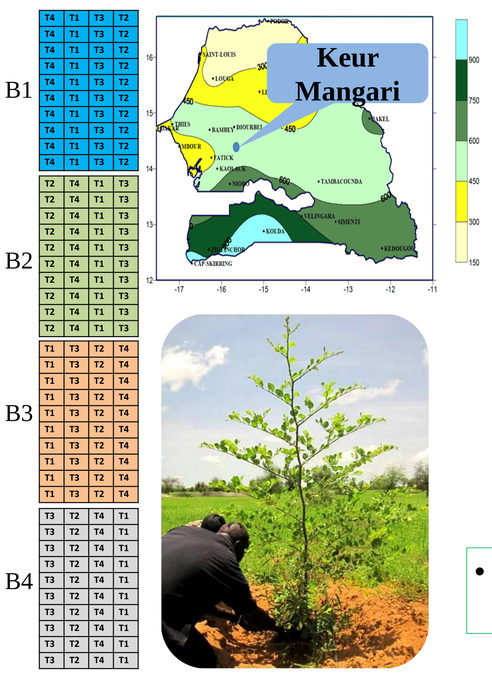
Treatment	Height (cm)	Collar diameter (mm)	Total dry biomass (g)	Mycorrhizal infection (%)
Tasset	15.2 ± 1.1 d	1.7 ± 0.2 c	0.9 ± 0.2 c	-
Tasset+Ri	33.6 ± 3.2 b	3.2 ± 0.3 b	1.3 ± 0.4 b	64.4 ± 6.1 b
Gola	23.4 ± 1.2 c	2.1 ± 0.1 c	1.7 ± 0.2 b	-
Gola+Ri	48.2 ± 2.2 a	4.2 ± 0.4 a	2.6 ± 0.5 a	70.2 ± 9.3 a
Provenance (P)	***	*	***	ns
Inoculation (I)	***	***	***	***
(P) × (I)	***	**	***	ns

Significant levels: *P<0.05; **P<0.01; ***P<0.001; ns=not significant, according Tukey's HSD

- Mycorrhizal treatments of plants had significantly-greater growth than non-inoculated. Gola provenance inoculated grew better than Tasset provenance inoculated.

Experiment in field conditions

Study site and experimental design



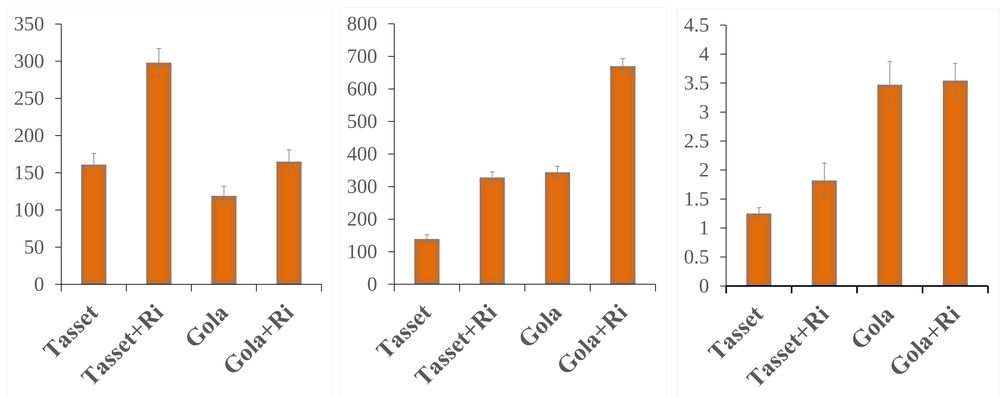
Effect of inoculation with *R. irregularis* IR27 on growth, rate of survival and mycorrhizal colonization of *Z. mauritiana* provenances 24 months after transplanting

Treatment	Height (cm)	Crown diameter (cm)	Mycorrhizal infection (%)	Rate of survival (%)
Tasset	127.7 ± 07.9 c	128.8 ± 10.0 c	22.6 ± 3.8 b	63.2 ± 10.0 b
Tasset+Ri	309.7 ± 13.2 a	280.1 ± 17.8 a	46.1 ± 5.6 a	82.5 ± 4.2 a
Gola	159.4 ± 20.1 b	168.3 ± 17.5 b	19.0 ± 5.1 b	55.0 ± 9.1 c
Gola+Ri	284.5 ± 20.8 a	295.3 ± 15.4 a	45.3 ± 8.8 a	80.0 ± 6.4 a
Provenance (P)	**	***	ns	ns
Inoculation (I)	***	***	***	*
(P) × (I)	**	**	ns	*

Significant levels: *P<0.05; **P<0.01; ***P<0.001; ns=not significant, according Tukey's HSD

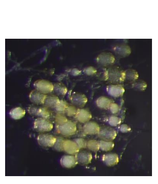
- Positive effects of inoculation with *R. irregularis* IR27 on growth, mycorrhizal infection and rate of survival of the two provenances of jujube trees.

Effect of inoculation with *R. irregularis* IR27 on fruit production of *Z. mauritiana* provenances 18 months after transplanting

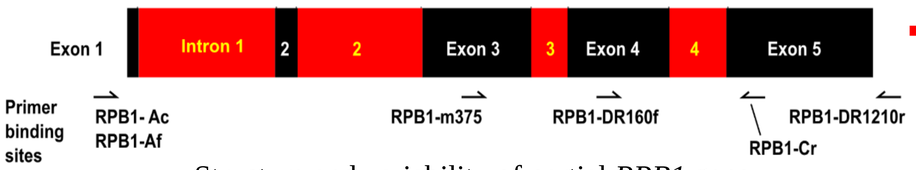


- Positive effects of inoculation with *R. irregularis* IR27 on fruit production of the two provenances of jujube trees

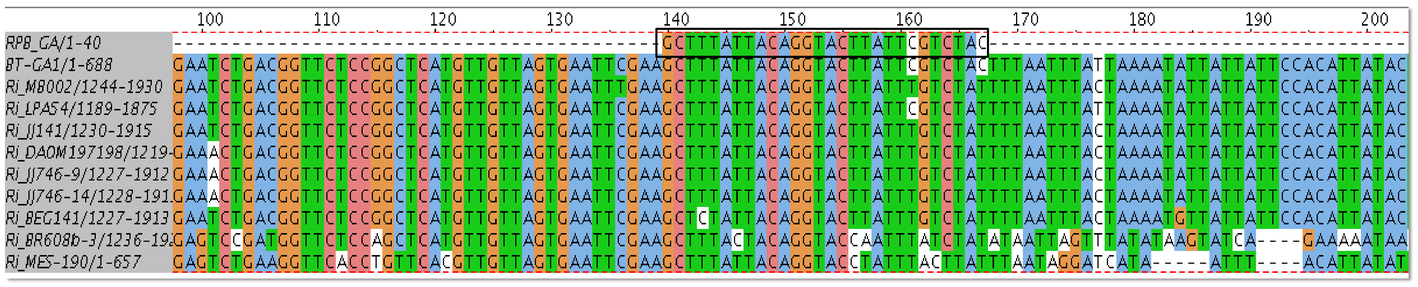
Tracing the inoculated *R. irregularis* IR27 in *Z. mauritiana* roots



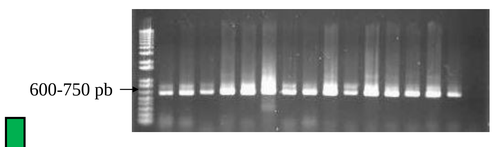
IR27



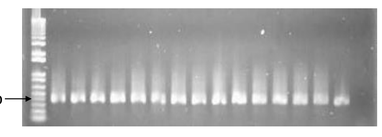
Structure and variability of partial *RPB1* gene



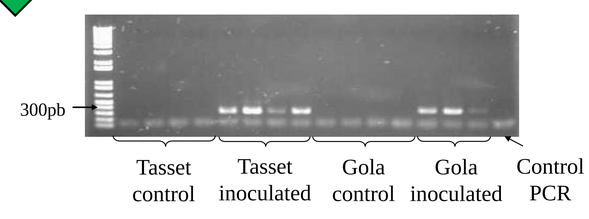
New specific primer **RPB_GA-F** for *R. irregularis* IR27 was designed by Amplify4 (version 1.0)



Detection of Eukaryotes with LR1/NDL22 primers

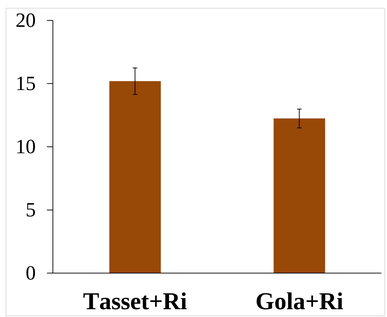


Detection of AM fungi with FRL3/FRL4 primers



Detection of IR27 with RPB_GA-F/RPB_DvT922 primers

Abundance of *R. irregularis* IR27 (%)



Quantification of root colonization by *R. irregularis* IR27 compared to the population of *Rhizophagus* in the field experiment

R. irregularis IR27 was specifically traced in inoculated roots by qPCR and still accounted for 12 to 15 % of the root colonization by *Rhizophagus*, 24 months after planting. Thus, RPB_GA-F/RPB_DvT922 primer is well suitable for tracking and quantification of *R. irregularis* IR27 in *Rhizophagus* communities associated to *Z. mauritiana* roots in the field. Overall, the results demonstrate that the ecological engineering strategy based on the use of *R. irregularis* IR27 is beneficial to jujube tree growth and fruit production in orchard. We have also shown that *R. irregularis* IR27, although still present 24 months after inoculation, is replaced by local species, and is not destroying the local mycorrhizal fungal community.

References

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[4] Guissou T, Sanon KB, Babana A, Bâ AM (2016) Effect of of arbuscular mycorrhizae on growth and mineral nutrition of greenhouse propagated fruit trees from diverse geographic provenances. Biotechnol. Agron. Soc. Environ. 20: 417-426.

Acknowledgements

LAPSE
LMI Adaptation des Plantes et microorganismes associés aux Stress Environnementaux
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